IMMUNOLOGIC EXPERIMENTS WITH PLATELETS OF HUMAN BLOOD

FRANK R. MENNE

From the John McCormick Institute for Infectious Diseases, Chicago, and the Department of Pathology, University of Oregon, Portland, Ore.

Marino [1] produced an antiserum in rabbits with platelets from the guinea-pig and found that this antiserum destroyed guinea-pig platelets, but was not toxic to the guinea-pig when injected intravenously. Le Sourd and Pagniez,[2] repeating the experiments of Marino, studied also the effect of antiplatelet serum on the clotting time of the corresponding blood, and concluded that the antiserum was lytic for platelets but caused no delay in the clotting time and was not toxic for guinea-pigs. These results were confirmed also by Chevel and Rogers.[3] Cole [4] separated the platelets from human blood, injected rabbits and demonstrated agglutination of the platelets with the rabbit antiserum, which had no effect on the clotting time of human blood. Sacerdotti [5] obtained an antirabbit platelet serum from the dog, and demonstrated its specificity and found it highly toxic in vivo. Injection of rabbits with the antiserum caused platelet reduction, hemorrhages and death in shock. Repeating this experiment, Stschastnyi [6] was unable to confirm the specificity of the serum. Aynaud [7] also disagreed with Sacerdotti and concluded that the antiserum was species specific but not cell specific, agglutinating and causing lysis of platelets in vitro, properties shared also by antiserum for red corpuscles. He was unable to demonstrate a specific effect of antiplatelet serum by absorption tests.

Ledingham and Aberd,[8] studying the relationship of antiplatelet serum to the so-called hemorrhagic diathesis in man, confirmed the power of platelet antiserum to bind complement and were unable to demonstrate this property in anti-erythrocytic serum. They obtained other results like those mentioned. The effects of subcutaneous and

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1 Compt. rend. Soc. de biol., 1905, 58, p. 194.
2 Compt. rend. Acad. de sc., 1906, 118, p. 562.
3 Compt. rend. Soc. de biol., 1907, 63, p. 501.
4 Bull. Johns Hopkins Hospital, 1907, 17, p. 261.
5 Arch. ital. biol., 1908, 52, p. 153.
6 Folia Serol., 1909, 2, p. 285.
7 Compt. rend. Soc. de biol., 1911, 70, p. 54.
8 Lancet, 1914, 186, p. 1673.

intravenous injections of antiguinea-pig platelet serum in guinea-pigs were striking; subcutaneous hemorrhages, phagocytosis and agglutination of red corpuscles and reduction in platelets. This work was repeated in more detail by Ledingham and Bedson,[9] who were unable to obtain any such results with antileukocytic serum and concluded that the action was due to a strong lytic and agglutinating power of antiplatelet serum. Lee and Robertson [10] confirmed these observations and pointed out that the action was dependent on complement, noting also that the serum of guinea-pigs with experimental purpura hemorrhagica had no effect on platelets in vivo or in vitro.

More recently, Bedson [11] demonstrated specific agglutination of rabbit antiserum of guinea-pig platelets by absorption tests controlled with antiserums for other blood elements. He concluded that the early results of Sacerdotti were correct and maintained that the antiplatelet serum is cell specific and that platelets are independent elements of the blood without genetic relationship with other blood cells. He further [12] showed by absorption tests that antiplatelet serum also can produce hemorrhagic purpura.

With the exception of Cole, none of the workers cited studied human blood elements, and it seemed advisable therefore to investigate the immunologic properties of the platelets of human blood.

PREPARATION OF ANTIGENS

Human platelets were obtained from the blood of patients with hypertension, bled for therapeutic purposes (Drs. Koessler, Bookbinder and Borsack). The usual method of fractional centrifuging was used for their separation. It was found almost impossible to remove all of the erythrocytes and leukocytes because of the strong tendency for compact clumping of the leukocytes and platelets, the lighter erythrocytes remaining in the supernatant platelet suspension. The latter would often appear free from red cells under the microscope, but by high speed centrifugation, a definite red mass of cells would form. The variation in size of platelets also affects their separation by this procedure so that even with great care a more or less impure antigen necessarily results. The platelets obtained as outlined were washed 5 times with salt solution; each cubic centimeter of the final suspension contained the platelets from 30 c c of blood.

[9] Ibid., 1915, 50, p. 311.
[10] Jour. Med. Research, 1916, 38, p. 323.
[11] Jour. Path. & Bacteriol., 1921, 24, p. 469.
[12] Ibid., 1922, 25, p. 94.

The leukocytes were obtained from the early exudate of gonorrheal arthritis (Drs. Herrold and Forester). Such exudates may yield perfect suspensions of apparently unchanged leukocytes, largely of the polymorphonuclear variety. The leukocytes were washed 5 times in large volumes of salt solution and then made into 10% suspension for injection. No bacteria could be found in the final suspension, although the whole exudate immediately after removal gave positive gonococcus cultures.

Erythrocytes and serum were obtained in the usual manner. The erythrocytes were washed six times with salt solution and suspended in salt solution.

PRODUCTION OF ANTISERUMS

The antiserums were produced as follows: The platelet suspension was injected intravenously in rabbits at 3-day intervals in doses of 1, 2, 4, 6, and 8 c c. Because of the strong tendency to clumping, the leukocytes were injected intramuscularly also at 3-day intervals in quantities of 1, 2, 3, 4, and 6 c c of a 10% suspension. Other rabbits injected intravenously with a 1:20 extract of leukocytes also produced an antileukocytic serum. The arythrocytes were given intravenously on 6 succeeding days in 1 c c doses of the solid corpuscles after centrifugation and human serum was given intravenously in 4, 8, 10, and 12 c c quantities also at 3-day intervals. The animals were bled on the fifth and tenth days after the last injection.

For precipitin tests platelet extract was made by suspending washed platelets in sterile distilled water in the proportion of 1:10 by weight; after shaking for several hours, an equal quantity of sterile 1.8% salt solution was added so that an extract of about 1:20 in 0.9 salt solution was obtained. The portion of the platelets that actually went into solution was of course not known. The subsequent dilutions of the centrifugated clear extract were made on the bases of the original proportion of platelets in distilled water.

Leukocytic extracts were made in a similar manner, and the subsequent dilutions were made on the basis of the original protion in distilled water. The erythrocytic extract was made by suspending carefully washed corpuscles of a definite quantity of blood in many times that quantity of sterile distilled water, then adding as much again of 1.8% salt solution and centrifuging thoroughly. In this way, clear solutions with 0.9 sodium chloride were obtained; 50 c c of this extract contained the corpuscles from 1.0 c c of blood. Extract of the stroma of red corpuscles was prepared by thoroughly washing the corpuscles and laking with about 5 times the quantity of distilled water, to which was added a small amount of ether to insure complete dissolution. To this solution an equal quantity of saturated ammonium sulphate was added. The resulting precipitate was filtered, washed with water and salt solution, ammonium sulphate again added and the washing repeated. The final dilution of the precipitate in 0.9 salt solution was one part of precipitate in 40 parts of salt solution. The clear portion removed after centrifuging was used for the precipitin test.

All precipitin tests were made by overlaying the antiserum with extract or serum and looking for a ring at the point of contact after one hour at room temperature.

To determine the agglutinin titer, the suspension of platelets used was similar to that injected. Dilutions of antiserums were added to this suspension. For agglutination test with erythrocytes a 2.5% (5% whole blood) suspension was used. The lytic titer was determined by the use of similar suspensions with the addition of 0.025 c c of fresh guinea-pig complement to each tube, the complement unit being determined by titration against a constant quantity—0.1 c c—of anti-erythrocytic serum. In both of these tests as well as that for opsonins the antiserums were inactivated first at 56 C for half an hour. For opsonic titration progressive dilutions of antiserum were added to mixtures of human corpuscles and dog leukocytes obtained from the pleural cavity 12 hours after an intrathroacic injection of sterile aleuronat. The leukocytes were drawn into a warm 2% citrate solution and washed with warm salt solution being being added to the erythrocytic suspension.

Table 1 is an illustrative record of the main results.

TABLE 1

TITRATIONS OF ANTIPLATELET AND OTHER ANTISERUMS

Antigen	Antiserum				Normal Rabbit Serum
	Platelet	Leugo-cytic	Erythro-cytic	Serum	
Precipitin Titers					
Platelet extract	200	0	100	0	0
Leukocytic extract	0	640	0	0	0
Extract of erythrocytic stroma	100	0	800	0	0
Erythrocytic extract	0	0	80	200	0
Serum	200	0	400	6400	0
Agglutinin Titers					
Platelets	1536	24	96	48	0
Erythrocytes	384	48	3072	768	0
Lytic Titers					
Platelets	24	0	0	0	0
Erythrocytes	24	0	1536	96	0
Opsonic Titers					
Human erythrocytes and dog leukocytes	768	0	1536	3072	0

The figures represent the highest dilutions of the antiserums at which the results were obtained in case of agglutination, lysis, and opsonification. In the case of the precipitin tests, the figures represent the highest dilutions of the antigens prepared as described in which a definite precipitate was obtained with the antiserums after one hour at room temperature.

In considering the precipitin titers of the various antiserums, it must be remembered that the solubility in water of the protein constituents of the blood cells are not known. Kaempler [13] quotes Fornio as stating that platelets are rich in thrombokinase which is yellow and water soluble, but he does not mention the extent of the solubility and no other information bearing on this point was found. In the light of these facts, the figures in table 1 may indicate a much higher specificity of reaction than they appear to do.

It is apparent that the leukocytic antiserum is strictly specific; the serum antiserum is also quite specific, reacting, however, with erythro-

[13] Am. Jour. Surg., 1915, 29, p. 401.

cytic extract in a 1:200 dilution, but this may be explained by the presence of a small amount of erythrocytic elements in human serum. The precipitates formed by bringing platelet antiserum in contact with extract of erythrocytic stroma and with human serum in low dilutions may be explained by the presence of some corpuscles in the platelet suspension used as antigen and then the serum undoubtedly contains some platelet elements in solution. Werbitski [14] claims to have extracted rabbit platelets with the serums of man and animals and to have studied the bactericidal properties of such extracts, but no other reference to the serum solubility of platelets has been found.

In order to test further the specificity of the platelet antiserum it was diluted 1:100 and thoroughly mixed with an equal quantity of normal human serum. After standing at room temperature for one hour this mixture was centrifugated and the clear solution tested with platelet extract: A definite precipitate formed at the junction point showing that the serum failed to remove the specific precipitin for platelet extract.

The agglutinin tests of the various antiserums also indicate specificity. In order to further substantiate this, 1 part of platelet antiserum was treated with 2 parts of a heavy suspension of platelets, the mixture being incubated at 37 C. for 2 hours and then centrifugated. The antiserum so treated agglutinated platelets in a dilution of 1:24 and erythrocytes in a dilution of 1:192, 1:384 being the titer of the untreated antiserum for erythrocytes. Similarly, platelet antiserum was mixed with 2 parts of a 25% suspension of human erythrocytes (50% human blood) in salt solution for 2 hours at 37 C. The treated antiserum now agglutinated platelets in a dilution of 1:768—about one half the original titer—and erythrocytes in a dilution of 1:1 only. While the platelets were able to remove the agglutinins from the platelet antiserum so that subsequent agglutination was reduced to a minimum, erythrocytes under the same conditions failed to remove the agglutinins for platelets.

The agglutinin titer for human leukocytes could not be determined accurately, because of the marked spontaneous clumping of leukocytes, and this phase of the work is to be taken up again later.[15]

[14] Ztschr. f. Hyg. u. Infektionskr., 1911, 68, p. 63.

[15] While this article was in preparation there appeared a report by Rosenthal and Falkenheim on agglutinins for different elements in human and chicken blood in which they conclude that the platelets are related more closely to the leukocytes than to the erythrocytes (Arch. f. exp. Path. u. Pharmakol., 1922, 92, p. 231).

Apparently the antiserums were not rich in lysins, and pronounced lytic effect was obtained with erythrocytes and erythrocytic antiserum only. A prompt clearing occurred of platelet suspension with low dilutions of platelet antiserum and no effect on platelets was noticed with other antiserums. Opinion appears to be divided as to the lytic action of platelet antiserum for platelets. As stated, complement fixation has been demonstrated by several, but my results with complement were not so definite or uniform as those obtained with agglutinin and precipitin tests.

It is interesting to note that phagocytosis of red corpuscles was not observed with antileukocytic serum, while platelet antiserum gave an opsonic titer of 768. The presence of opsonins in the platelet antiserum may be explained as the result of the antigenic effect of erythrocytes in the platelet suspension injected as antigen.

The reactions described are in harmony with the results Bedson obtained with guinea-pig blood. Evidently platelets contain specific antigens, as might be expected from the morphologic observations of Wright [16] and others as to their origin from megakaryocytes in marrow.

SUMMARY

Immunological reactions of human platelets indicate the presence in platelets of specific antigenic constituents, and results of the precipitin test point to a definite difference in the constitution of the platelets and the leukocytes in human blood. The observations on human platelets support the views of Bedson and others that the platelets differ in their constitution from other elements of the blood.

[16] Boston Med. and Surg. Jour., 1906, 154, p. 643.